

Product usage

Before using this product, please read the Limited Use statement below

Important Limited Use information for pTiGer4-SEAP

The purchase of the pTiGer4-SEAP vector conveys to the buyer the non-transferable right to use the purchased amount of the product and components of the product in research conducted by the buyer (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes.

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TECHNICAL SUPPORT

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pTiGer4-SEAP

A multigenic plasmid for inducible SEAP expression, selectable with Puromycin

Catalog code: ptg4-sp

<https://www.invivogen.com/tet-on-ptiger-reporter>

For research use only

Version 24A16-NJ

PRODUCT INFORMATION

Contents:

- 20 µg of pTiGer4-SEAP provided as lyophilized DNA
- 1 ml of Puromycin (10 mg/ml)

Storage and Stability:

- Product is shipped at room temperature.
- Lyophilized DNA should be stored at -20 °C.
- Resuspended DNA should be stored at -20 °C and is stable for up to 1 year.
- Store Puromycin at 4 °C or at -20 °C. The expiry date is specified on the product label.

Quality control

- Plasmid construct is confirmed by restriction analysis and full-length open reading frame (ORF) sequencing.
- After purification by ion exchange chromatography, predominant supercoiled conformation is verified by electrophoresis.

PRODUCT DESCRIPTION

InvivoGen provides a family of plasmids featuring a tetracycline-inducible reporter gene. The pTiGer4-SEAP plasmid encodes the secreted embryonic alkaline phosphatase (SEAP) and the Puromycin resistance marker for selection in both mammalian cells and bacteria. This plasmid can be used as a transfection control for plasmids of the pTiGer-mcs family.

The SEAP expression is only possible upon transfection of cells featuring the tetracycline repressor (TetR) protein¹, such as InvivoGen's HEK-RepTor™ or A549-RepTor™ cells. These cells express TetR constitutively in the nucleus, where it binds to tetracycline operator (tetO) sequences and represses gene transcription. Upon incubation with doxycycline (a synthetic tetracycline derivative), TetR is released from the tetO sequences and the SEAP-encoding gene is transcribed.

pTiGer-Lucia and pTiGer-eGFP plasmids are also available.

PLASMID FEATURES

SEAP expression cassette

- **hCMV enh/ hEF1 prom:** This composite promoter combines the human cytomegalovirus (HCMV) enhancer and the core promoter of the human elongation factor-1 α (EF-1 α)².
- **tetOtetO:** This sequence is also known as TRE (Tetracycline Response Element). It is a repeat of the 19-nucleotide sequence of the tetracycline operator (tetO)¹.
- **SEAP:** The secreted embryonic alkaline phosphatase catalyzes the hydrolysis of pNitrophenyl phosphate (pNpp), producing a yellow end product. SEAP levels can be evaluated qualitatively with the naked eye, and quantitatively using a spectrophotometer in combination with SEAP detection media, such as HEK-Blue™ Detection, or QUANTI-Blue™ Solution, a SEAP detection reagent.

- **SV40 pAn:** The Simian Virus 40 late polyadenylation signal enables efficient cleavage and polyadenylation reactions resulting in high levels of steady-state mRNA³.

Puromycin antibiotic selection cassette

- **pMB1 Ori:** This minimal *E. coli* origin of replication with the same activity as the longer Ori.
- **hAldA enh/ hFerH prom:** This composite promoter combines the human aldehyde dehydrogenase (aldA) enhancer and the core promoter of the human ferritin heavy chain gene (FerH).
- **EM7:** This bacterial promoter enables the constitutive expression of the *pac* gene in *E. coli*.
- **pac:** The resistance to Puromycin is conferred by the *pac* gene from *Streptomyces* which encodes a N-acetyl-transferase. The *pac* gene is driven by the human AldA/FerH promoter in tandem with the bacterial EM7 promoter allowing selection in both mammalian cells and *E. coli*.
- **mEF1 5'UTR:** The 5'UTR (untranslated region) of the murine EF-1 α enhances *pac*-encoding mRNA stability and protein translation.
- **hβGlo pAn:** The human β -Globin pAn is a strong polyadenylation signal placed downstream of *pac*⁴.

1. Hillen, W., Wissmann, A. (1989). Tet repressor-tet operator interaction. Protein-Nucleic Acid Interaction. DOI: 10.1007/978-1-349-09871-2_7. 2. Kim DW. et al., 1990. Use of the human elongation factor 1 α promoter as a versatile and efficient expression system. Gene 91(2):217-23. 3. Carswell S. & Alwine JC., 1989. Efficiency of utilization of the simian virus 40 late polyadenylation site: effects of upstream sequences. Mol Cell Biol. 9(10):4248-58. 4. Yu J. & Russell JE., 2001. Structural and functional analysis of an mRNP complex that mediates the high stability of human β -globin mRNA. Mol Cell Biol. 21(17):5879-88.

METHODS

Plasmid resuspension

- Quickly spin the tube to pellet the DNA.
- To obtain a plasmid solution at 1 µg/µl, resuspend the DNA in 20 µl of sterile water.
- Store the resuspended plasmid at -20 °C.

Plasmid amplification and cloning

Plasmid amplification and cloning can be performed in *E. coli* GT115 or other commonly used laboratory *E. coli* strains, such as DH5 α .

Puromycin usage

Puromycin can be used at 100-125 µg/ml in *E. coli* in liquid or solid media and at 1-10 µg/ml to select Puromycin-resistant mammalian cells.

Generation of Tet-inducible expression cells

For a general procedure using InvivoGen's RepTor™ cell lines, please visit: <https://www.invivogen.com/reptor-cells>.

RELATED PRODUCTS

Product	Description	Cat. Code
Puromycin	Selection antibiotic	ant-pr1
QUANTI-Blue™	SEAP detection reagent	rep-qbs

TECHNICAL SUPPORT

InvivoGen USA (Toll-Free): 888-457-5873

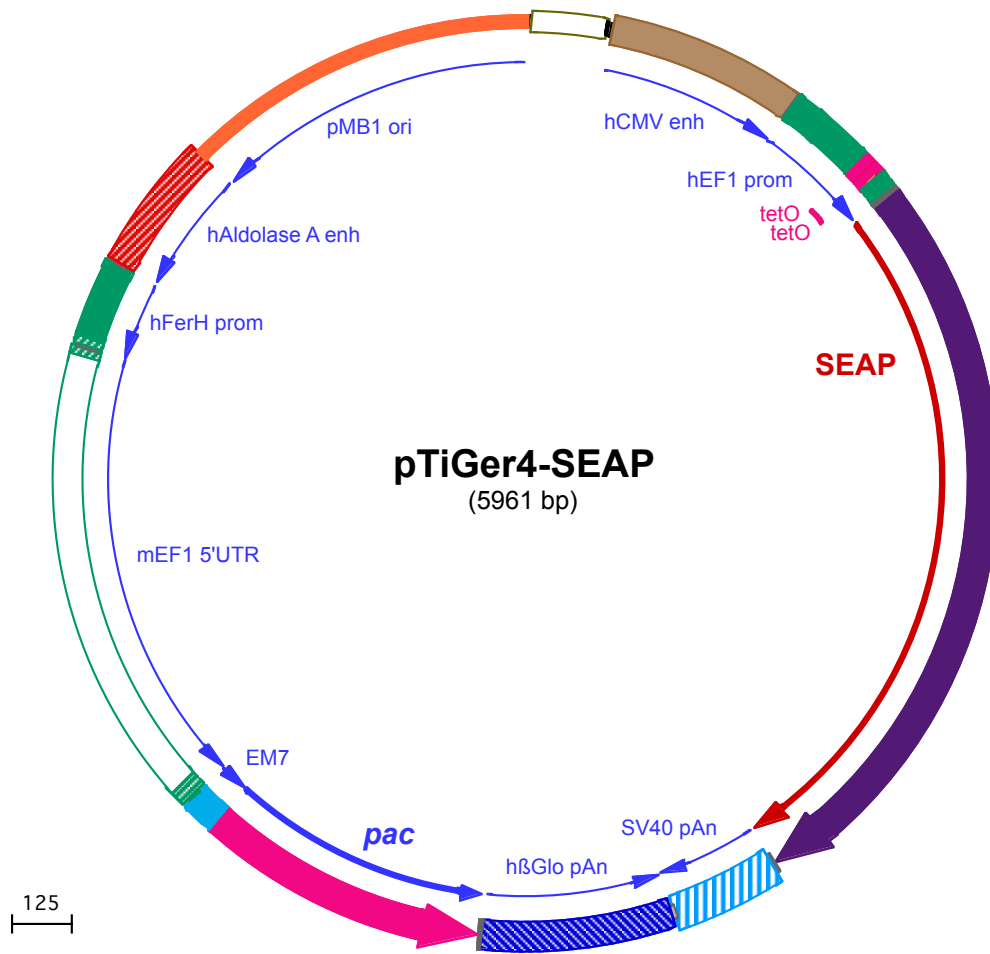
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1 CTCGAGCGGCCGCAATAAAATATCTTTATTTTCATTACATCTGTGTGTTGGTTTTTTGTGTGAATCGTAACTAACATACGCTCTCCATCAAAACAAAACG
101 AAACAAAACAAACTAGCAAAATAGGCTGTCCCAAGTCAAGTGCAGGTGCCAGAACATTTCTCTATCGAAGGACCTGCAGGCGTTACATAACTTACGGTA
201 AATGGCCCCGCTGGTGACCGCCCAACGACCCCGCCATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCATTGACGTC
301 AATGGGTGGAGTATTTACGGTAACTGCCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCC
401 CGCCTGGCATTATGCCCAGTACATGACCTTATGGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCATGATGATGCGGTTTTGG
501 CAGTACATCAATGGGCGTGGATAGCGGTTTACTCACGGGATTTCCAAGTCTCCACCCATTGACGTCAATGGGAGTTTGTGTTTACTAGTCAGTGGCC
601 AGAGCGCACATCGCCACAGTCCCCGAGAAGTTGGGGGAGGGGTCCGCAATTGATCCGGTGCCTAGAGAAGTGGCGCGGGTAACTGGGAAAGTGAT
701 GTCGTGTAAGTGGCTCCGCTTTTTCCGAGGGTGGGGGAGAACCCTATATAAGTGCAGTAGTTGCCGTGAACGTTTCCCTATCAGTGATAGAGATCTCCC
801 TATCAGTGATAGAGATCTTTCGCAACGGGTTTCCGCCAGAACACAGCTGAAGCTTACCAGGTCACCATGGTTCTGGGCGCCTGCATGCTGCTGCTGCTG
120 M V L G P C M L L L L
901 CTGCTGCTGGCCTGAGGCTACAGCTCTCCCTGGGCATCATCCAGTTGAGGAGGAGAACCAGGACTTCTGGAACCGCGAGGCAGCCGAGGCCCTGGGTG
120 L L L G L R L Q L S L G I I P V E E E N P D F W N R E A A E A L G
1001 CCGCAAGAAGCTGCAGCCTGCACAGACAGCCGCAAGAACCTCATCATCTTCTGGGCGATGGGATGGGGGTGTCTACGGTACAGCTGCCAGGATCCT
45 A A K K L Q P A Q T A A K N L I I F L G D G M G V S T V T A A R I L
1101 AAAAGGGCAGAAGAAGGCAAACTGGGGCTGAGATACCCCTGGCTATGGACCGTTCATATGTGGCTCTGTCCAAGACATACAATGTAGACAAACAT
78 K G Q K K D K L G P E I P L A M D R F P Y V A L S K T Y N V D K H
1201 GTGCCAGACAGTGGAGCCACAGCCACGGCTACCTGTGCGGGTCAAGGGCAACTCCAGACCATTGGCTTGTAGTGCAGCCGCGCTTAAACAGTGA
112 V P D S G A T A T A Y L C G V K G N F Q T I G L S A A A A R F N Q C
1301 ACACGACACGCGCAACGAGGTATCTCCGTGATGAATCGGGCCAAGAAAGCAGGGAAGTCAAGTGGGAGTGGTAACCACCACAGTGCAGCACGCTC
145 N T T R G N E V I S V M N R A K K A G K S V G V V T T T R V Q H A S
1401 GCCAGCCGGCACCTACGCCACAGGTGAACCGCAACTGGTACTCGGACGCCAGCTGCCTGCCTCGGCCCGCAGGAGGGTGCAGGACATCGCTACG
178 P A G T Y A H T V N R N W Y S D A D V P A S A R Q E G C Q D I A T
1501 CAGCTCATCTCCAACATGGACATTGATGTGATCCTGGTGGAGGCCGAAAGTACATGTTTCGCATGGGAACCCAGACCCTGAGTACCAGATGACTACA
212 Q L I S N M D I D V I L G G G R K Y M F R M G T P D P E Y P D D Y
1601 GCCAAGGTGGGACCAGGTGGACGGGAAGAATCTGGTGCAGGAATGGTGGCGAAGCGCCAGGGTGCCTGATGTGTGAACCGCACTGAGCTCATGCA
245 S Q G G T R L D G K N L V Q E W L A K R Q G A R Y V W N R T E L M Q
1701 GGCTTCCCTGGACCCGTCTGTGACCCATCTCATGGTCTCTTTGAGCCTGGAGACATGAAATACGAGATCCACCGAGACTCCACACTGGACCCCTCCCTG
278 A S L D P S V T H L M G L F E P G D M K Y E I H R D S T L D P S L
1801 ATGGAGATGACAGAGGCTGCCCTGCGCCTGCTGAGCAGGAACCCCGGGCTTCTTCTCTCTGTTGGAGGGTGGTGCATCGACCACGGTATCACGAAA
312 M E M T E A A L R L L S R N P R G F F L F V E G G R I D H G H H E
1901 GCAGGGCTTACCGGGCACTGACTGAGACGATCATGTTGACGACGCCATTGAGAGGGCGGGCCAGCTCACCAGCGAGGAGGACACGCTGAGCCTCGTAC
345 S R A Y R A L T E T I M F D D A I E R A G Q L T S E E D T L S L V T
2001 TGCCGACCATCCACGCTTCTTCTCGGAGGCTACCCCTGCGAGGAGCTCCATCTTCCGGCTGGCCCTGGCAAGGCCGGACAGGAAGGCTAC
378 A D H S H V F S F G G Y P L R G S S I F G L A P G K R A P A
2101 ACGGTCTCTATACGAAACGGTCCAGGCTATGTGCTCAAGGACGGCGCCCGGGGATGTTACCGAGAGCGAGAGCGGGAGCCCGAGTATCGGCAGC
412 T V L L Y G N G P G Y V L K D G A R P D V T E S E S G S P E Y R Q
2201 AGTCAGCAGTGCCTGGACGAAGAGACCCACGAGGCGAGGAGCTGGCGGTGTTCCGCGCGGCCCGAGGCGACCTGGTTCACGGCGTGCAGGAGCA
445 Q S A V P L D E E T H A G E D V A V F A R G P Q A H L V H G V Q E Q
2301 GACCTTATAGCGCAGTGCATGGCTTCCGCGCTGCTGGAGCCCTACACCGCTGCGACCTGGCGCCCCCGCGGCACCACCGACCGCGCACCCG
478 T F I A H V M A F A A C L E P Y T A C D L A P P A G T T D A A H P
2401 GGGCGTCCCGTCCAAGCGTCTGGATTGAAGCTAGCTGGCCAGACATGATAAGATACATTGATGAGTTGGACAAACCACAACACTAGAATGCAGTAAAA
512 G R S R S K R L D •
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93 P G I E A F V A G A E V S E P T T W V A V A A G D D A V W V K G I
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59 D L G V R T L F L E Q L E T V R E I H R D P D V T H R T A P Y D A F
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